STRATEGIC DIAGNOSTICS INC.
EnviroGard® Cyclodienes Plate Kit
73310

Intended Use
The EnviroGard Cyclodienes Plate Kit is a quantitative laboratory test for the detection of cyclodiene residues in water.

Test Principles
The EnviroGard Cyclodienes Plate Kit uses polyclonal antibodies which bind both cyclodienes and a chlordane-enzyme conjugate. Cyclodienes in the sample compete with chlordane-enzyme conjugate for a limited number of antibody binding sites. Antibodies which bind cyclodienes are immobilized to the inside of the test wells. In the assay procedure you will:

Since the same number of antibody binding sites are available in every well, and each well receives the same number of chlordane-enzyme conjugate molecules, a sample containing a low concentration of cyclodienes allows the antibody to bind many chlordane-enzyme conjugate molecules. The result is a dark blue solution.

Conversely, a high concentration of cyclodienes allows fewer chlordane-enzyme conjugate molecules to be bound by the antibodies, resulting in a lighter blue solution.

NOTE: Color is inversely proportional to cyclodiene concentration.

Darker color = Lower concentration
Lighter color = Higher concentration

Performance Characteristics
The EnviroGard Cyclodienes Plate Kit test does not differentiate between various cyclodiene com-pounds, but detects their presence to differing degrees. The following table shows the value for 50% Bo* and the approximate value for the Lower Limit of Detection (LLD). All concentrations are in parts per billion (ppb).

Compound	50% Bo	90% Bo (LLD)
Aldrin	84	17
Chlordane	30	5
Dieldrin	27	2
Endosulfan	6	0.6
Endrin	3	0.15
Heptachlor	33	4

*%B_O equals the average optical density (OD) of the calibrator or sample divided by the average OD of the negative control multiplied by 100 (see "Calculate the Results").

Precautions
- Store all kit components at 4°C to 8°C (39°F to 46°F) when not in use.
- Do not freeze plate kit components or expose them to temperatures greater than 37°C (99°F).
- Allow all reagents and samples to reach ambient temperature (18°C to 27°C or 64°F to 81°F) before you begin the test.
- Do not store plate kit components for more than 8 hours at ambient temperature.
- Do not use kit components after the expiration date.
- Do not mix reagents or test well strips from plate kits with different lot numbers.
- Use approved methodologies to confirm any positive results.
- Do not dilute or adulterate test reagents or use samples not called for in the test procedure; this may give inaccurate results.
- Tightly recap the 10 microgram/milliliter (µg/mL) Chlordane stock solution after use to prevent evaporative losses.
- Some solutes and particulates found in untreated ground or surface waters may affect the sensitivity level of this kit.
- If you test something other than water, use a calibrator with a matrix comparable to your sample.

Materials Provided in the EnviroGard Cyclodienes Plate Kit
This test kit contains the following items:

8 strips of 12 wells each, antibody-coated, in strip holder
1 vial of Negative Control (0.0 ppb Chlordane)
1 vial of 10 µg/mL chlordane (10,000 ppb) in methanol
1 vial of Chlordane-Enzyme Conjugate
1 vial of Substrate
1 vial of Chromogen
1 vial of Stop Solution

Materials You Provide
- Marking pen
- Positive displacement pipette capable of measuring 10 µL (microliters)
- Disposable-tip pipette which will measure 80 µL, 200 µL, 250 µL, 750 µL, 800 µL, and 990 µL
- 100 mL volumetric flask
- Three 1 mL glass tubes
- Microtiter plate reader
- Tape or Parafilm®
- Watch or timer
- Clean running water or a wash bottle containing tap or deionized water [500 milliliters (mL)]
- Deionized water (or the equivalent) for calibrator preparation
- Calculator (optional)
- Orbital shaker (optional)
- Microtiter plate washing device (optional)
- Multi-channel pipette (optional)

Calibrator Preparation
NOTE: Because chlordane tends to adhere to glass and plastic surfaces once in aqueous solution, prepare calibrators just prior to running the assay.

The EnviroGard Cyclodienes Plate Kit contains a 10 µg/mL stock solution in methanol. **Do not use the stock solution directly in the assay**. This stock solution **must** be diluted in laboratory grade water to prepare working calibrators, at levels of 5 ppb, 25 ppb, and 100 ppb.

NOTE: Accurate pipetting of the stock solution and thorough mixing of the calibrator solution are critical to the performance of this assay.

1. Be certain that the 10 µg/mL chlordane stock solution is at room temperature. Gently swirl the vial to mix before pipetting.
2. Using a 10 µL positive displacement pipette, transfer 10.0 µL of the 10,000 ppb stock solution to a clean glass tube which contains 990 µL of lab grade water. Vortex to mix thoroughly. Label this tube “100 ppb Chlordane”.
3. Transfer 250 µL of the 100 ppb solution to a clean glass tube which contains 750 µL of lab grade water. Vortex to mix thoroughly. Label this tube “25 ppb Chlordane”.
4. Transfer 200 µL of the 25 ppb solution to a tube which contains 800 µL of lab grade water. Vortex to mix thoroughly. Label this tube “5.0 ppb Chlordane”.

Assay Procedure
Have your plate kit materials available and follow these steps:

NOTE: The raised markings on the strip holder help keep the format in the correct order while you add the reagents and sample. When adding reagents, hold the dropper vial upright over the wells and allow the drops to fall freely into the wells. *Do not* touch the dropper tip to the sides of the wells. To add calibrators and samples, a pipette must be used.

1. Plan the strip format allowing for the placement of the negative control (C), 3 calibrators (C1-C3), and the samples (S1-8).

	1	2	3	4	5	6	7	8	9	10	11	12
A	C	C	C1	C1	C2	C2	C3	C3	S1	S1	S2	S2
B	S3	S3	S4	S4	S5	S5	S6	S6	S7	S7	S8	S8
C												
D												

NOTE: To set up an assay using fewer than eight strips, remove the unneeded strips and store them at 4°C to 8°C (39°F to 46°F) in the re-sealable plastic bag (with desiccant) provided.

2. Add 80 µL of Negative Control (C), 80 µL of each calibrator (C1-C3), and 80 µL of each sample (S1 to S8) to their respective wells, as shown above.
3. Using the same order of addition, add 2 drops (80 µL) of Chlordane-Enzyme Conjugate to each well.

NOTE: If you are running more than three strips, it is recommended that a multi-channel pipette be used in steps 2, 3, 7, 8 and 10.

4. Thoroughly mix the contents of the wells by moving the strip holder in a rapid circular motion on the benchtop for about 1 minute. Be careful not to spill the contents!
5. Cover the wells with tape or Parafilm to prevent evaporation and incubate at ambient temperature for 1 hour. Orbital mixing at 200 rpm during incubation is preferable, but not mandatory.
6. After incubation, carefully remove the covering and vigorously shake the contents of the wells into a sink. Flood the wells completely with cool running tap water, then shake to empty. Repeat this wash step five times. Invert the plate and tap out as much water as possible. Alternatively, use a microtiter plate washer for the wash steps.
7. Add 2 drops (80 µL) of Substrate to each well, beginning with the Negative Control (C) and Calibrators (C1 to C3), and finishing with the Samples (S1 to S8).
8. Add 2 drops (40 µL) of Chromogen to each well except the blank in the same order as for Substrate. The chromogen solution contains methanol; therefore, each drop is equal to 20 µL rather than 40 µL.

NOTE: You MUST add substrate before the chromogen. However, if all 8 strips are to be used at once, you can premix the substrate and chromogen by combining the contents of the 2 vials. Add 120 µL of this mixture to each well. Mix immediately before use and do not retain any of the unused mixture.

9. Mix the contents of the wells, as in step 4. Cover the wells with new tape or Parafilm and incubate at ambient temperature for 30 minutes. Orbital mixing at 200 rpm is preferable, but not mandatory.

WARNING: Stop Solution is 2.5 N sulfuric acid.

10. Add 1 drop (40 µL) of Stop Solution to each well to arrest the blue color development and turn the reaction solution yellow. Mix thoroughly, without spilling, until all of the blue has converted to yellow.

Interpret The Results

Spectrophotometric Measurement and Analysis

1. Adjust the wavelength of your microtiter plate reader to 450 nanometers (nm). (If it has dual wavelength capability, use 600 or 650 nm as the "reference" wavelength.)
2. If the plate reader does not auto-zero on air, zero the instrument against 120 µL water in a blank well, then measure and record the optical density (OD) of each well. Or, measure and record the OD in every well, then subtract the OD of the water blank from each reading.
3. If the microtiter plate reader you are using has data reduction capabilities, use a semi-log curve fit for the standard curve. You can also calculate the results as described in the next section.

Calculate the Results

1. After you read all of the wells, average the OD of each set of calibrators and samples, and calculate the %Bo as follows:

$$\%B_o = \frac{\text{average OD of calibrator or sample}}{\text{average OD of negative control}} \times 100$$

The %Bo calculation is used as a means of equalizing different runs of an assay. While the raw OD readings of negative controls, calibrators, and samples are likely to differ from run to run, the %Bo relationship of calibrators and samples to the negative control should remain fairly constant.
2. Graph the %Bo of each calibrator against its cyclodiene concentration on a semi-log scale (see "Sample Calculations").
3. Determine the cyclodiene concentration of each sample by finding its %Bo value and the corresponding concentration level on the graph (or plug the %Bo values into the equation of the line calculated by linear regression).
4. If the %Bo of a sample is greater than that of the lowest calibrator, the sample should be reported as "less than 5 ppb". If the %Bo of a sample is less than that of the highest calibrator, the sample should be reported as "greater than 100 ppb" or the sample should be diluted with laboratory grade water so it falls on the standard curve when the assay is repeated.

Ordering Information

Description	Catalog Number
EnviroGard Cyclodienes Plate Kit	73310

Technical Assistance

Strategic Diagnostics Inc.
111 Pencader Drive
Newark, Delaware 19702-3322 USA
800-544-8881
302-456-6789 Phone
302-456-6782 Fax
www.sdix.com
techservice@sdix.com

General Limited Warranty

Strategic Diagnostics Inc. (SDI) warrants the products manufactured by it against defects in materials and workmanship when used in accordance with the applicable instructions for a period not to extend beyond a product's printed expiration date. SDI MAKES NO OTHER WARRANTY, EXPRESSED OR IMPLIED. THERE IS NO WARRANTY OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE.

3099880, Rev 3MAR98